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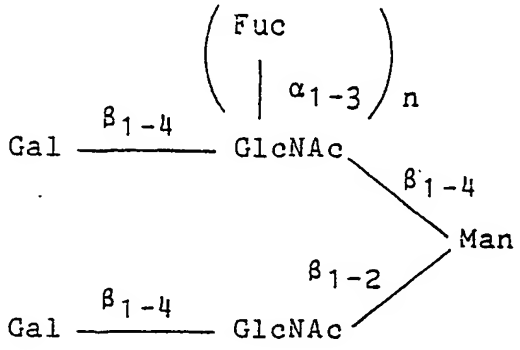
(54) Monoclonal antibody and method for the production thereof.

(57) A monoclonal antibody specific for an α_1-acid glycoprotein or for at least one antigenic determinant included in a sugar chain of the following formula:



wherein Gal means galactose, GlcNAc means N-acetylglucos-amine, Man means mannose, Fuc means fucose, and n is 0 or 1, which is useful for the measurement of glycoproteins in cells, tissues and blood and therefore is useful for diagnosis of various diseases, particularly tumors, and a method for the production thereof by fusing a neoplasm cell line with antibody-producing cells from an animal which has been immunized against an desialated glycoprotein and culturing the resultant hybridoma.

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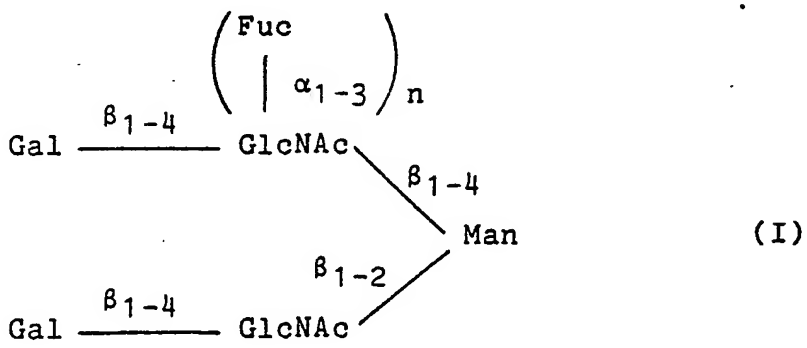
MONOCLONAL ANTIBODY AND METHOD FOR THE PRODUCTION THEREOF

This invention relates to a monoclonal antibody and a method for the production thereof. More particularly, it relates to a monoclonal antibody specific for an α_1-acid glycoprotein or specific for at least one antigenic determinant included in a sugar chain of the following formula which is contained in glycoproteins such as α_1-acid glycoprotein:



(I)

wherein Gal means galactose, GlcNAc means N-acetylglucosamine, Man means mannose, Fuc mans fucose, and n represents 0 or 1 (hereinafter, this sugar chain is referred to as "sugar chain-I")

and a method for the production of the monoclonal antibody. The monoclonal antibody is useful for the measurement of glycoproteins in cells, tissues and blood and therefore useful for diagnosis of various diseases.

Prior Art

A monoclonal antibody is usually prepared by fusing antibody-producing cells from a mammal which has been immunized against a particular antigen, with an appropriate neoplasm cell line having unlimited growing properties, for example, a myeloma cell line, and cloning the resultant hybridoma [cf. G. Köhler and C. Milstein, Nature, 256, 495 (1975)]. The hybridoma inherits an antibody producing property from the antibody-producing cells and also unlimited growing property from the neoplasm cell line. Each antibody-producing cell can produce an antibody having single specificity (monoclonal antibody), and hence, the hybridoma prepared therefrom can produce the monoclonal antibody in a medium within a flask or in ascites or blood of the host animals. The monoclonal antibody thus prepared has homogeneity and high specificity, and hence, is particularly useful for diagnosing test.

It has recently been known that the concentration of α_1-acid glycoprotein (hereinafter, referred to as "α_1-AGP") in cells, tissues or blood has a relation with various diseases such as tumors. For instance, it is known that human α_1-AGP is an acute phase reactant having a molecular weight of about 44,000 and the blood level thereof increases in patients suffering from heavy infectious disease or other various diseases. Moreover, it is reported that α_1-AGP suppresses an immunoreaction (e.g. lymphocyte blastogenesis, etc.) in vitro [cf. M. Bennett, et al., Proc. Natl. Acad. Sci. , USA, 77 (10) 6109 (1980)].

Besides, recent attention has focused on α_1-AGP as an immunosuppressive material which is found in blood of patients suffering from tumors, and Ishida et al have reported that a subfraction of α_1-AGP having an isoelectric point of about 3.0 was isolated from ascites of a tumor patient, which was named as Immunosuppressive Acidic Protein - (abbreviated as "IAP"), and that it could be used as a tumor marker by measuring the IAP level in human blood with an antiserum prepared from rabbit immunized thereby [cf. K. Tamura, et al., Cancer Res., 41, 3244 (1982)]. Although this IAP isolated by Ishida et al. is usually used as a tumor marker which is non-specific for organs, the anti-IAP antiserum used in the test is a mixture of various antibodies having various specificities (polyclonal antibody) and does not have high specificity. Furthermore, this IAP has also a problem that antibody titer or specificity of the an-

tiserum varies depending on the animal individual to be immunized. In the diagnosis of tumors, an attention has also focused on monoclonal antibody, but there has never been known a monoclonal antibody specific for α_1-AGP or specific for sugar chain-I.

Brief Summary of the Invention

The present inventors have studied on an monoclonal antibody specific for α_1-AGP or for at least one antigenic determinant included in the sugar chain-I and found that when a hybridoma is prepared by using cells of an animal immunized merely against α_1-AGP or a glycoprotein containing sugar chain-I, the desired monoclonal antibody can not be obtained, but when the terminal sialic acid moiety of sugar chain in α_1-AGP or glycoproteins containing the sugar chain-I is deleted, such desialated glycoproteins show high immunogenicity, and hence, by fusing a neoplasm cell line with cells of animals immunized against the desialated glycoproteins, there can be obtained the desired hybridoma which can produce the desired monoclonal antibody specific for α_1-AGP or for at least one antigenic determinant included in the sugar chain-I.

An object of the invention is to provide a monoclonal antibody specific for α_1-AGP or for at least one antigenic determinant included in the sugar chain-I. Another object of the invention is to provide a method for producing a monoclonal antibody specific for α_1-AGP or for at least one antigenic determinant included in the sugar chain-I by fusing a neoplasm cell line with antibody producing cells from an animal which has been immunized against an de-sialated glycoprotein and culturing the resultant hybridoma. These and other objects and advantages of the invention will be apparent to persons skilled in the art from the following description.

Brief Description of the Drawing

The accompanying Fig. 1 shows a calibration curve by ELISA (enzyme-linked immunosorbent assay) wherein a monoclonal antibody, HA-7 of this invention was immobilized. Fig. 2 shows a calibration curve by ELISA wherein a monochlonal antibody, HA-7 of this invention was immobilized and biotin-conjugated HA-5 was further reacted. Fig. 3 shows a calibration curve by ELISA wherein a monoclonal antibody, HA-13 of this invention was immobilized and biotin-conjugated HA-13 was further reacted.

Detailed Description of the Invention

In the present invention, the animals are immunized against α_1-AGP or glycoproteins containing sugar chain-I which has been subjected to a treatment for deletion of sialic acid. The glycoproteins containing sugar chain-I include α_1-AGP, fetuin, ceruloplasmin, CEA (carcino embryonic antigen), and the like. Among these glycoproteins, a presence of the sugar chain both with a fucose (n = 1 in the formula I) and without a fucose (n = 0 in the formula I) has been known for α_1-AGP, ceruloplasmin and CEA. A presence of the sugar chain with a fucose (n = 1 in the fomula I) has not been reported for fetuin.

The deletion of sialic acid from α_1-AGP and other glycoproteins can be carried out by conventional methods, for example, hydrolysis with an enzyme (e.g. sialidase), or hydrolysis with an acid. The enzyme for the deletion of sialic acid is preferably free from protease and highly pure. Impure sialidase usually includes protease and hence disadvantageously hydrolyzes not only sialic acid but also peptide chain. The hydrolysis with an acid should be done under the conditions that any peptide chain is not hydrolyzed but sialic acid is deleted as much as possible. For example, it can be done by treating the glycoproteins with a diluted mineral acid (e.g. diluted sulfuric acid) under heating at a temperature of about 80°C for several hours.

Immunization of animal is carried out by injecting an emulsion mixture of the de-sialated glycoprotein with Freund's complete adjuvant. The Freund's complete adjuvant is mixed in order to make sure the immunization, and other conventional means is also usable.

The animal to be immunized includes various animals, but is preferably mice and rats, from which there are obtained many neoplasm cells suitable as a partner of the hybridoma , in particular, BALB/c mouse from which many neoplasm cells are available.

The antibody-producing cells are most preferably spleen cells of the above BALB/c mouse, but may be other animal cells, such as spleen cells of rats, lymphocytes of rabbits, lymphocytes of sheep, and the like.

The neoplasm cells may be any cells which can give unlimited growing property to the hybridoma, but are preferably myeloma cells. Suitable examples of the myeloma cell lines are P_3-X63-Ag8 [cf. Nature, 256, 495 (1975)], P_3-X63-Ag8-653 [ATCC number: CRL-1580, cf. J. Immunol., 123, 1548 (1979)], P_3-NS1/1-Ag4-1 [cf. Eur. J. Immunol., 6, 511 (1976)], S194, Y3, SP2/0 [ATCC

number: CRT-1581, cf. Nature, 276, 269 (1978)], MPC-11 [ATCC number: CCL-167, cf. J. Exp. Med., 131 , 515 (1970)], and mutants of these cells. These cells lack nucleic acid producibility by salvage pathway and is preferable also in view of selection of suitable hybridoma as mentioned hereinafter. Among the abovementioned cell lines, P_3-NSI/1-Ag4-1 has a high growth rate and a high antibody producing capacity and hence is used preferably.

The fusion of the antibody-producing cells and the neoplasm cells can be done by a known technique, for instance, by using HVJ (Hemagglutinin Virus of Japan, other name: Sendai virus, cf. Yoshio Okada, "Cell fusion and Cell Engineering" issued by Kodansha, page 19 (1975)] or polyethylene glycol [cf. V.T. Ohi, L.A. Herzenberg, "Selected Method in Cellular Immunology" edd. by B.B. Michell, issued by W. H. Freeman, Chapter 17] or by electric fusion, or the like.

The mouse myeloma cells as mentioned above lack the nucleic acid producibility by salvage pathway, and hence can synthesize nucleic acid only by de novo pathway. On the other hand, the hybridoma cell line has a nucleic acid producibility by salvage pathway origined from the antibody-producing cells and hence can selectively be grown in a hypoxanthine-aminopterine-thymidine - (HAT) medium (usually, RPMI-1640 medium supplemented with 10 -15 % fetal calf serum (FCS).

The production of a monoclonal antibody specific for glycoproteins by the hydridoma can be confirmed by agglutination reaction using sheet erythrocyte which is bound with de-sialated glycoprotein or untreated glycoprotein. It can also be confirmed by an enzyme linked immunosorbent assay (ELISA) using an immobilized glycoprotein which is de-sialated or untreated

The hybridoma which produces a monoclonal antibody specific for glycoproteins is cloned by a method of limiting dilution.

The hybrodoma cell line thus obtained can be grown in vitro on a suitable medium (usually, RPMI-1640 medium supplemented with 10 -15% FCS) while producing the desired antibody. The hybridoma cell line may also be cultured within the body of the same animal as that from which the neoplasm cells used for the cell fusion are obtained, by which the desired monoclonal antibody can be produced in the ascites and blood in a high concentration.

The monoclonal antibody may be used for the detection of glycoproteins in cells or sera in the form of a culture supernatant or ascites or serum, but it may be purified by a conventional purification method, such as salting out with ammonium sulfate, ion exchange chromatography, affinity chromatography, and the like. The purified antibody may be used as it is, or after being labelled with radioisotope, fluorescene, enzyme, biotin, etc.

The detection can be done by known techniques, such as radioimmunoassay, fluorescence immunoassay, enzyme immunoassay, agglutination, and the like. Detection of glycoproteins in serum is preferably carried out by agglutination, radioimmunoassay, or enzyme immunoassay. Detection of glycoproteins in cells and tissues is preferably carried out by fluorescence immunoassay and enzyme immunoassay. The monoclonal antibody of this invention is not limited to these usages.

The monoclonal antibody of this invention is useful for the detection of glycoproteins in cells, tissues and blood and hence for diagnosis of various diseases, particularly diagnosis of tumors.

The present invention is illustrated by the following Example and Reference Example.

Example

(1) Preparation of de-sialated α_1-AGP by the treatment with sulfuric acid:

Purified α_1-AGP was obtained from pooled human sera by a known method [cf. W. Burgi and K. Schmid, J. Biol. Chem, 236, 1066 (1961)]. The α_1-AGP (10 mg) was dissolved in 0.1N sulfuric acid - (10 ml), and the mixture was stirred at 80°C for 1 hour. After cooling, the reaction mixture was neutralized with 3N aqueous NaOH solution, and then dialyzed against water at 4°C. The resultant was lyophilized to give de-sialated α_1-AGP (about 4 mg).

(2) Preparation of de-sialated fetuin by the treatment with sulfuric acid:

A commercially available fetuin (Type IV, manufactured by Sigma Co., purified product obtained from fetal calf serum) was treated in the same manner as described in the above (1) to give a de-sialated fetuin.

(3)-(a) Preparation of de-sialated α_1-AGP by the treatment with enzyme:

The same α_1-AGP (10 mg) as used in the above (1) was dissolved in PBS (phosphate buffered saline, pH 7.4) (10 ml), and thereto was added sialidase [E.C.3.2.1.18] (0:25 unit), and the

mixture was stirred at 37°C for 30 minutes. The reaction mixture was dialyzed against water at 4°C and then lyophilized to give a de-sialated α_1-AGP - (about 4 mg).

(3)-(b) Preparation of de-sialated ceruloplasmin by the treatment with enzyme:

A commercially available ceruloplasmin (manufactured by Green-Cross Co., Japan) was treated in the same manner as described in the above (3)-(a) to give de-sialated ceruloplasmin.

(4) Preparation of antibody producing cells:

The de-sialated α_1-AGP obtained in the above - (1) was dissolved in physiological saline solution, sterilized by filtration, mixed with Freund's complete adjuvant, and the mixture was injected intraperitoneally to mouse (BALB/c) in an amount of de-sialated α_1-AGP: 500 μg/mouse, by which the mouse was immunized. After 3 weeks, the mouse was boosted with a mixture of an aqueous aluminum phosphate solution and a physiological saline solution of the de-sialated α_1-AGP in the same amount as above.

Third day after the booster injection, the spleen cells were taken out and were used as antibody producing cells for the following example.

(5) Cell fusion:

The antibody producing cells (6.5×10^8 cells) were fused with neoplasm cells (P_3-NSI/1-AG4-1) - (3.3×10^8 cells) by polyethylene glycol - (PEG#1500). The fused cells were cultured in HAT medium (HAT-containing RPMI-1640 medium supplemented with 15 % FCS) to grow selectively only the hybridoma. About 10 days after the cell fusion, the specific antibody was checked by agglutination test using an antigen-sensitized sheep erythrocyte. The specific antibody producing hybridoma was cloned by limiting dilution, and then, there were selected 5 hybridoma cell lines which produced monoclonal antibody specific for α_1-AGP (these cell lines and the monoclonal antibodies produced from each cell line are designated as HA-2, HA-3, HA-5, HA-7 and HA-10, respectively) and one hybrodoma cell line which produced monoclonal antibody specific for an antigenic determinant included in the sugar chain-I (the cell line and the monoclonal antibody produced from the cell line is designated as HA-13). These hybridoma cell lines were unlimitedly grown in vitro (in RPMI-1640 medium supplemented with 15 % FCS) and also within peritoneal cavity of BALB/c mouse and hence the desired monoclonal antibody could be obtained from the culture supernatant or the ascites.

(6) Detection of specificity of antibody by Western blotting:

(i) Hybridoma cell lines, HA-2, HA-3, HA-5, HA-7 and HA-10:

α_1-AGP, de-sialated α_1-AGP and human serum were subjected to an electrophoresis with sodium laurylsulfate-polyacrylamide gel (SDS-PAGE, gel concentration 10 %), and thereafter, an electric blotting was carried out from the gel to nylon membrane (Zeta-Probe, manufactured by Bio-Rad Co.).

The nylon membrane treated with 10 % sheep serum-containing PBS and then reacted with a culture supernatant of a hybridoma cell line which produced anti-α_1-AGP monoclonal antibody, and further reacted with a horse radish peroxidase - (HRP)-anti-mouse Ig antibody, and thereafter, the reaction mixture was specifically stained by diaminobenzidine in the presence of H_2O_2.

By the above test, in the culture supernatant of all of the hybridoma cell lines in this invention, only the band of α_1-AGP (untreated and de-sialated) was specifically stained, by which it was confirmed that the monoclonal antibody of this invention can specifically react only with α_1-AGP.

(ii) Hybridoma cell line HA-13:

α_1-AGP, de-sialated α_1-AGP, fetuin, de-sialated fetuin, ceruloplasmin, and de-sialated ceruloplasmin were subjected to an electrophoresis with sodium laurylsulfate-polyacrylamide gel (SDS-PAGE, gel concentration 10 %), and thereafter, an electric blotting was carried out from the gel to nylon membrane (Zeta-Probe, manufactured by Bio-Rad Co.).

The nylon membrane was treated with 10 % sheep serum-containing PBS and then reacted with a culture supernatant of a hybridoma cell line (HA-13) which produced anti-sugar chain-I monoclonal antibody and further reacted with a horse radish peroxidase (HRP)-anti-mouse Ig antibody, and thereafter, the reaction mixture was specifically stained by diaminobenzidine in the presence of H_2O_2.

By the above test, the de-sialated α_1-AGP, de-sialated fetuin, and de-sialated ceruloplasmin were specifically stained, but untreated α_1-AGP, untreated fetuin and untreated ceruloplasmin were not stained, by which it was confirmed that HA-13 was specifically reacted with at least one antigenic determinant included in the sugar chain-I common to these glycoproteins.

(7) Characteristics of the monoclonal antibody and purification thereof:

(i) Class of the antibody:

It was confirmed that the immunoglobulins produced by the hybridoma cell lines of this inventioon are IgG, (HA-3, HA-7, HA-10) and IgM (HA-2, HA-5, HA-13), respectively, by Ouchterlony method [cf. O, Ouchterlony, Prog. Allergy, 5, 1 (1958)].

(ii) Purification by salting out with ammonium sulfate:

The culture supernatants or ascites obtained by culturing of the hybridoma cell lines were subjected to precipitation by saturation with 50 % ammonium sulfate, which was repeated twice, and thereafter, the precipitates were dialyzed against water at 4°C and lyophilized to give monoclonal antibody Ig fraction.

(iii) Purification by affinity chromatography:

The culture supernatants or ascites obtained by culturing of the hybridoma cell lines of this invention were subjected to the purification as they stand, or after being specifically bound with a de-silated α_1-AGP -Sepharose [which was prepared from CNBr-Sepharose (manufactured by Pharmacia, Sweden) and de-sialated α_1-AGP by a known method], followed by eluting the bound monoclonal antibody with a glycine-HCl buffer (pH 2.5) and then neutralizing. The solution was dialyzed against water at 4°C, and lyophilized to give affinity-purified monoclonal antibodies.

(8) Detection of α_1-AGP and an antigenic determinant included in the sugar chain-I in serum:

(i) Preparation of a monoclonal antibody bound with biotin:

The affinity-purified monoclonal antibody (2 mg) obtained above was dissolved in 0.1 mM aqueous $NaHCO_3$> solution (2 ml) and thereto was added a solution (200 µl) of NHS-biotin - (manufactured by Pierce Co.) (1 mg/ml) in dimethylformamide (DMF), and the mixture was reacted at room temperature for 4 hours. The reaction mixture was dialyzed against PBS at 4°C to give a monoclonal antibody bound with biotin.

(ii) Detection of α_1-AGP in serum by ELISA method-1:

A PBS solution of affinity-purified monoclonal antibody (0.05 mg/ml) was put on Falcon 3912 microtest plate in an amount of each 50 µl/well, and allowed to stand at room temperature overnight in order to immobilize the antibody. Thereafter, 5 % FCS-containing PBS was poured thereto in an amount of each 300 µl/well, and allowed to stand 37°C for 2 hours, by which non-specific adsorption site was blocked. A serum to be tested and a 5 % FCS-containing PBS solution of a known amount of α_1-AGP (for calibration) were added in each 25 µl/well, and it was allowed to stand at 37°C for 1 hour. A 5 % FCS-containing PBS solution of IgG fraction of rabbit anti-α_1-AGP antiserum (manufactured by Dako Co.) (0.01 mg/ml) was added in each 50 µl/well, and it was allowed to stand at 37°C for 1 hour. Furthermore, 5 % FCS-containing PBS solution (0.002 mg/ml) of HRP-labelled anti-rabbit IgG-antiserum (manufactured by Amersham) was added in an amount of each 50 µl/well, and it was allowed to stand at 37°C for 1 hour. The resultant was reacted with 1 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in the presence of H_2O_2, and a transmission at 409 nm was measured.

From the transmission in the well added with an α_1-AGP of a known concentration and the concentration thereof, a calibration curve was drawn. Based upon the calibiration curve, the concentration of α_1-AGP in the test serum was calculated.

An example of the calibration curve obtained by using HA-7 in the above procedure is shown in the accompanying Fig. 1.

In case of HA-7, the concentration of α_1-AGP was 1.1 ± 0.1 (S.E.) µg/ml in normal subjects (N = 10) and 2.2 ± 0.7 (S.E.) µg/ml in tumor patients (N = 13). Thus, it is clear that the α_1-AGP concentra-

tion in serum of the tumor patients was higher than that of the normal subjects and because of varying of the data depending on the state of disease, the data were widely distributed.

(iii) Detection of α_1-AGP in serum by ELISA method-2:

A PBS solution of an IgG fraction of a rabbit anti-α_1-AGP anti-serum (manufactured by Dako Co.) (0.1 mg/ml) was immobilized on a plate in the same manner as described in the above (8)-(ii).

In the same manner as in the above (8)-(ii), the non-specific adsorption site was blocked, and thereto were added a test serum and α_1-AGP having a known concentration. Thereafter, a 5 % FCS-containing PBS solution of biotin-conjugated monoclonal antibody (0.005 mg/ml) prepared in (8)-(i) and further a Tris buffered saline solution (TBS) - (pH 7.4) of HRP-labelled avidine (manufactured by Vector Co.) were added thereto, and it was reacted at 37°C for 15 minutes and then with ABTS like in (8)-(ii).

By the above method, a similar calibration curve to Fig. 1 was obtained. Based on the calibration curve, the α_1-AGP concentration in the test serum was calculated. As a result, in case of HA-2, the concentration of α_1-AGP was 1.68 ± 0.50 (S.E.) µg/ml in normal subjects (N = 10) and 1.58 ± 0.73 (S.E.) µg/ml in tumor patients. It was difficult to distinct HA-2 between the normal subjects and the tumor patients, but α_1-AGP in serum could clearly be detected.

(iv) Detection of α_1-AGP in serum by ELISA method-3:

In the same manner as described in (8)-(ii), an affinity-purified monoclonal antibody was immobilized onto a plate, the non-specific adsorption site was blocked, and the test serum and an α_1-AGP having a known concentration were reacted. Thereto was added biotin-conjugated monoclonal antibody was added, HRP-labelled avidine was bound and it was reacted with ABTS like in (8)-(iii). A calibration curve was drawn and based on the calibration curve, the α_1-AGP concentration in test serum was calculated.

The accompanying Fig. 2 shows a calibration curve obtained in case of immobilization of HA-7, followed by reacting with biotin-conjugated HA-5. By the result of the test, in case of using HA-7 and biotin-conjugated HA-5, the concentration of α_1-AGP was 0.37 ± 0.04 (S.E.) µg/ml in normal subjects (N = 10) and 0.84 ± 0.16 (S.E.) µg/ml in tumor patients (N = 13).

(v) Detection of an antigenic determinant included in the sugar chain-I in serum by ELISA method:

A PBS solution of affinity-purified monoclonal antibody (HA-13, 0.05 mg/ml) was poured onto Falcon 3912 microtest plate in an amount of each 50 µl/well, and the plate was allowed to stand at room temperature overnight to immobilize the antibody. Thereto was added 5 % FCS-containing PBS in each 300 µl/well, and it was allowed at 37°C for 2 hours, by which the non-specific adsorption site was blocked. Thereto were added a test serum and a 5 % FCS-containing PBS solution of de-sialated α_1-AGP having a known concentration in an amount of each 25 µl/well, and it was allowed to stand 37°C for 1 hour. At 5 % FCS containing PBS solution of biotin-conjugated monoclonal antibody - (biotin-HA-13) (0.002 mg/ml) prepared in the above (8)-(i) was added thereto, and further a TBS solution (Tris buffered saline solution, pH 7.4) of HRP-labelled avidine (manufactured by Vector Co.) - (0.002 mg/ml) was added, and it was reacted at 37°C for 15 minutes. Thereafter, it was reacted with 1 mM ABTS in the presence of H_2O_2, and a transmission at 409 nm was measured.

From the transmission of the well added with de-sialated α_1-AGP having a known concentration and concentration thereof, a calibration curve was drawn, and based on the calibration curve, the relative concentration of an antigenic determinant included in the sugar chain-I in the test serum was calculated.

One example of the calibration curve in this case is shown in the accompanying Fig. 3. The relative concentration of an antigenic determinant included in the sugar chain-I in serum was 12.2. ± 3.4 (S.E.) µg/ml in normal subjects (N = 10) and 4.8 ± 1.4 (S.E.) µg/ml in tumor patients (N = 13). Thus, it is clear that the serum of the tumor patients showed lower concentration of an antigenic determinant included in the sugar chain-I than that of the normal subjects, and hence, the abnormal glycometabolism in tumor patients can be detected.

(9) Detection of α_1-AGP and an antigenic determinant included in the sugar chain-I in cells:

Human lymphocytes (10^6 cells) were centrifuged (1,200 r.p.m., 5 minutes), washed with PBS, and thereto was added a PBS solution (5 ml) of biotin-conjugated monoclonal antibody (5 µg/ml) as prepared in (7)-(i), and it was reacted at 4°C for 2 hours. Then, a $NaHCO_3$ buffered saline solution (pH 8.2) (0.25 ml) of a fluorescein-labelled avidine - (manufactured by Vector Co.) (5 µg/ml) was added,

and it was reacted at 4°C for 30 minutes. The reaction mixture was washed with an ice-cooled PBS and then dispersed in a 50 % glycerin/PBS, and it was observed with a fluorescent microscope. As a result, there was specifically observed a fluorescence of fluorescein on the cell membrane of the specific lymphocytes.

(10) Detection of α_1-AGP and an antigenic determinant included in the sugar chain-I in tissues:

A tissue to be stained was fixed with Bouin solution or Carnoy solution, dehydrated and then embedded with paraffin. The paraffin-embedded tissue was cut in thin sections with a microtome and attached onto a slide glass. Paraffin was removed off with xylene-alcohol subsequently, and the tissue was washed by dipping in PBS for 10 mintues. In case of a freshly frozen sections, it was fixed with acetone at -20°C and then immediately washed with PBS.

A 50 % sheep serum-containing PBS was reacted with the tissue section prepared above at room temperature for 30 minutes, by which the non-specific binding site was blocked. The tissue thus reacted was further reacted with a 1 % sheep serum-containing PBS solution of a monoclonal antibody purified in the same manner as described in (7) (5 -25 μg/ml) or with a culture supernatant of monoclonal antibody-producing cells at room temperature for1 hour. The resultant was washed by dipping in PBS for 10 minutes, and then reacted with a 50 % sheep serum-containing PBS solution of an HRP-labelled sheep anti-mouse IgG antibody (10 -25 μg/ml), which had been adsorbed with human serum, at room temperature for 30 minutes. Moreover, after washing by dipping in PBS for 10 minutes, the tissue section thus reacted was specifically stained by diaminobenzidine in the presence of H_2O_2. After washing by dipping in PBS for 10 minutes, the tissue was further subjected to a counter-stain with methylene blue. The stained tissue was washed with water, dehydrated with alcohol-xylene subsequently and then mounted with a commercially available mounting agent, and it was observed with a microscope.

Examples of the stained tissues as above are as follows:

In case of HA-2 and HA-5, cytoplasm of epithelial cells in stomach, bile-bladder, gullet, etc., and liver cells was mainly stained, from which it is clear that α_1-AGP is distributed within the cytoplasm of these tissues. In case of HA-13, the epithelioglandular Brush border of these tissues was specifically stained, from which it is clear than an antigenic determinant included in the sugar chain-I is specifically distributed within this region. In case of HA-7, the tumor cells of mastocarcinoma tissue was stained in two patients (among five patients), but in the remaining three patients suffering from benign mastadenoma, it was entirely not stained. Besides, in patients suffering from renal cancer, ovarian cancer and uterus cancer, the tissues were stained, but the tissues of gastrointestinal cancer, lung cancer and thyrophyma were entirely not stained. Thus, HA-7 can be used for staining specifically a specific tumor tissue, and hence it is useful for diagnosis of tumors.

Reference Example

Mice were immunized with an untreated α_1-AGP in the same manner as described in the above (4), and the antibody-producing cells were taken out.

The antibody-producing cells (2.5×10^8 cells) were fused with neoplasm cells (P_3-NSI/1-Ag4-1) (1.1×10^8 cells) in the same manner as described in the above (5), and there was determined the presence of specific antibody in the culture supernatant of the resulting hybridoma by an enzyme immunoassay using immobilized α_1-AGP.

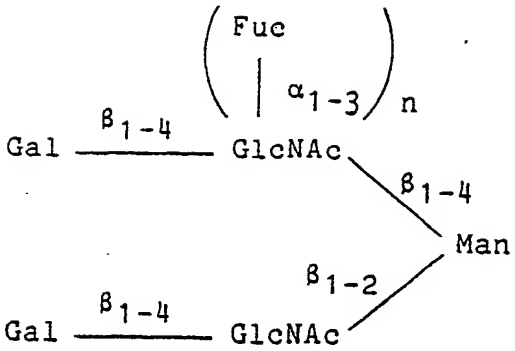
In all of 348 wells (100 %) poured with the cells, the hybridoma was grown, among which the antibody production was obversed in 25 wells (7 %) by the enzyme immunoassay. However, 10 of these hybridoma disappeared the antibody value before cloning step.

Colonies obtained from 15 strains cloned by limiting dilution showed no antibody titer by the enzyme immunoassay. Thus, it is difficult to obtain a stable antibody-producing hybridoma cell line when the animal is immunized with an untreated α_1-AGP.

Claims

1. A monoclonal antibody specific for α_1-acid glycoprotein.

2. A monoclonal antibody specific for at least one antigenic determinant included in a sugar chain of the formula:

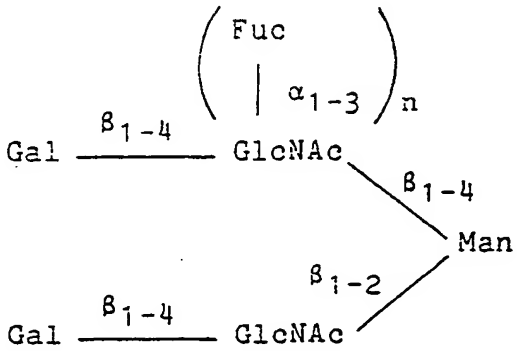


wherein Gal means galactose, GlcNAc means N-acetylglucosamine, Man means mannose, Fuc means fucose, and n represents 0 or 1.

3. A monoclonal antibody according to claim 2, wherein n is 0.

4. A method for the production of a monoclonal antibody specific for α_1-acid glycoproteins, which comprises fusing a neoplasm cell line with antibody-producing cells from an animal which has been immunized against an de-sialated α_1-acid glycoprotein, and culturing the resultant hybridoma.

5. A method for the production of a monoclonal antibody specific for at least one antigenic determinant included in a sugar chain of the formula:



wherein Gal means galactose, GlcNAc means N-acetylglucosamine, Man means mannose, Fuc means fucose, and n is 0 or 1, which comprises fusing a neoplasm cell line with antibody-producing cells from an animal which has been immunized against a de-sialated glycoprotein containing a sugar chain of the above formula, and culturing the resultant hybridoma.

6. A method according to claim 5, wherein n is 0.

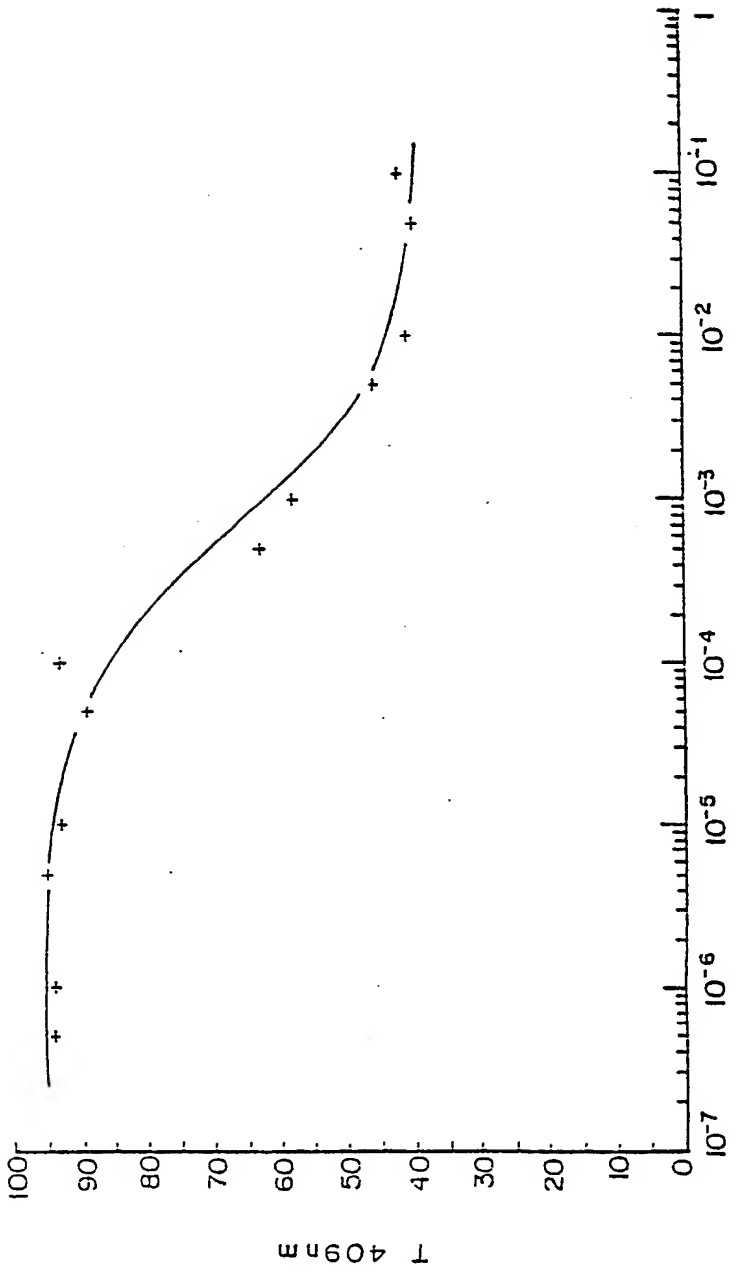
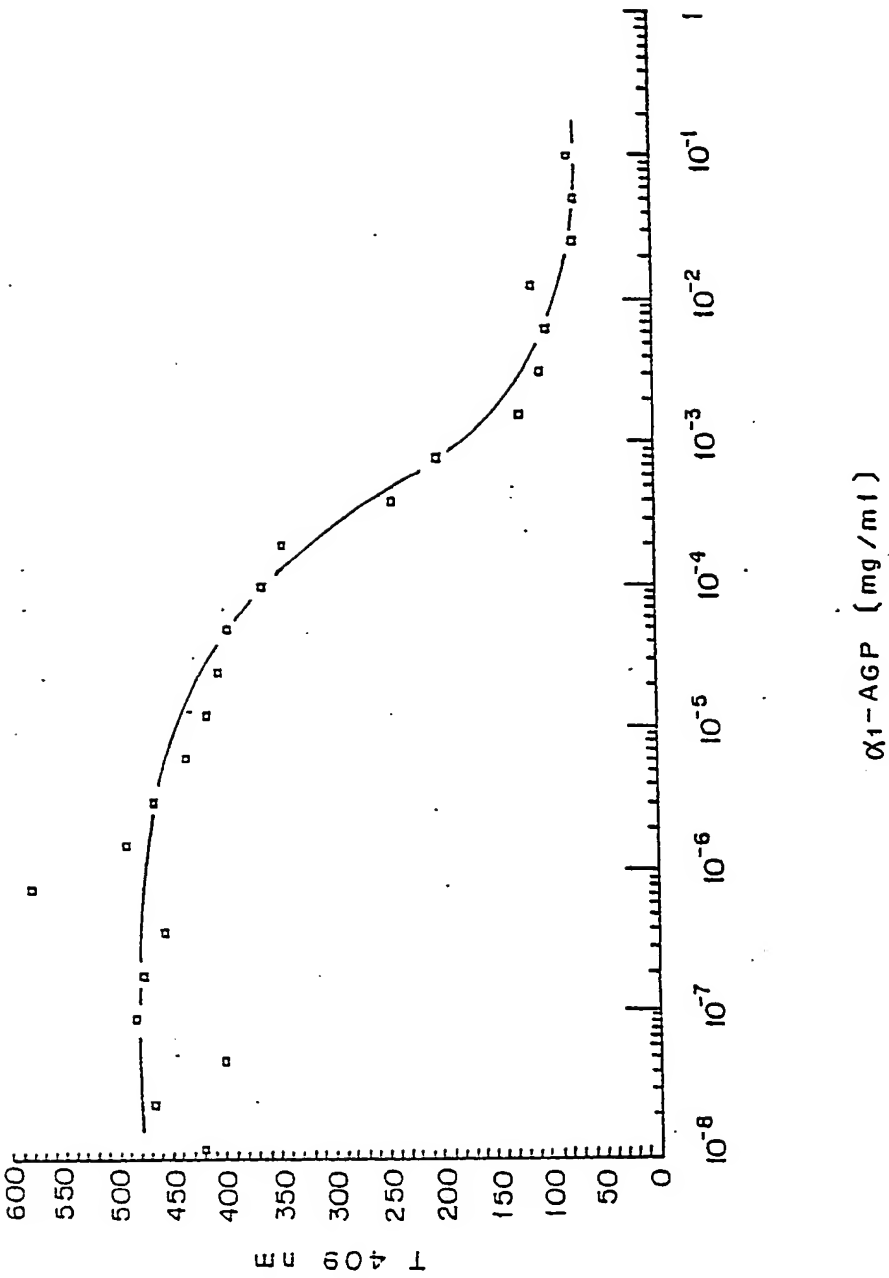

T 409nm
100
90
80
70
60
50
40
30
20
10
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
1
di-AGP [mg/ml]

Fig. 1

Fig. 2


600
550
500
450
400
350
300
250
200
150
100
50
0
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
1
α1-AGP (mg/ml)

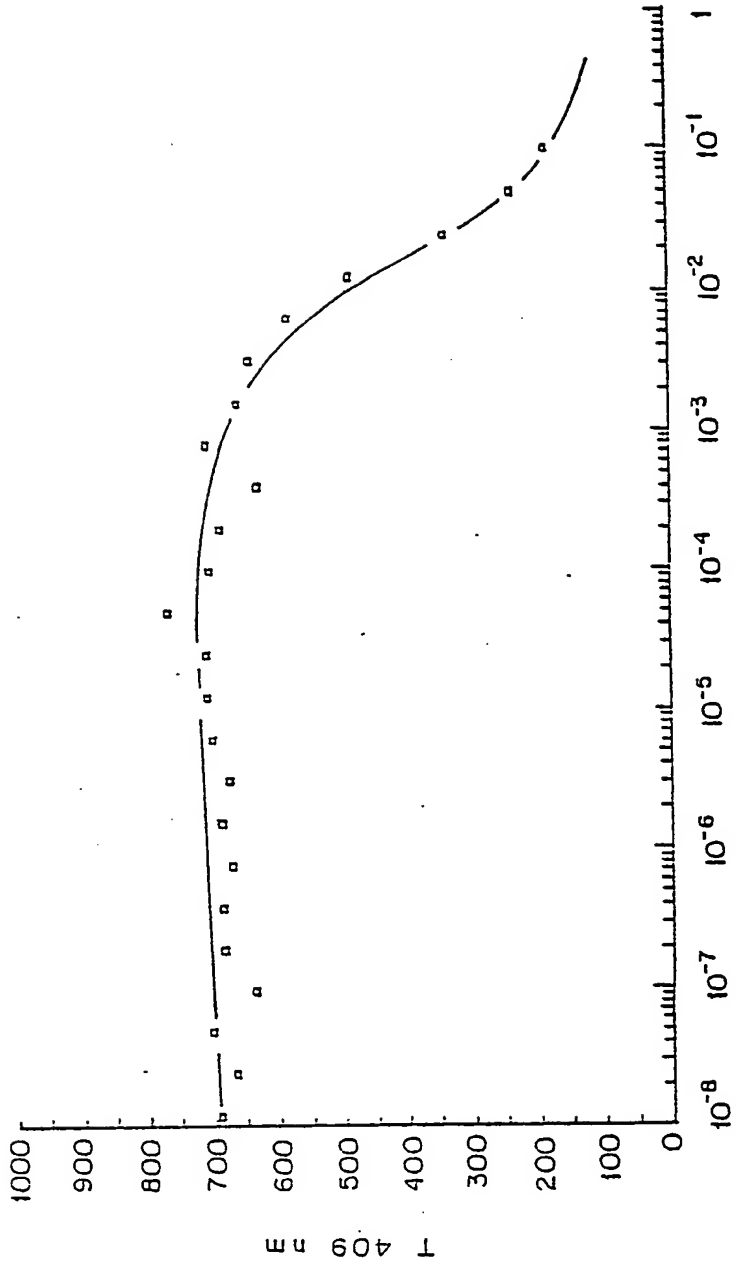

T 409 nm
1000
900
800
700
600
500
400
300
200
100
0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
1
α_1-AGP (mg/ml)

Fig. 3